

Composition Studies on Tobacco. XIV

Steam-Volatile, Neutral Substances in Various Types and Grades¹

D. Burdick, I. Schmeltz, R. L. Miller and R. L. Stedman

1914

Eastern Regional Research Laboratory, United States Department of Agriculture
Philadelphia 18, Pennsylvania, U.S.A.

This report describes a comparative study of certain steam-volatile, neutral substances in cigarette tobacco types and in aromatic or aroma-deficient grades of bright tobacco. In a series of sixteen publications, Onishi *et al.* (1955-1958) have presented an exhaustive composition study of the steam-volatile compounds in (mainly) flue-cured leaves using classical procedures of isolation and identification. Weybrew and Stephens (1962) have surveyed the carbonyl composition of the steam-volatile neutral fraction in various tobacco types and grades by a gas chromatographic method. The present study was designed to augment these investigations.

Method

The following method was used in the comparisons of types and grades (Figure 1): One hundred g of tobacco (50-60 mesh) were steam distilled, using distilled water as a source of steam. One thousand ml of distillate were collected in a vessel cooled in ice water. The distillate was saturated with sodium chloride and the solution was extracted three times (150 ml each) with redistilled ether. The pooled ether extract (A) was extracted 3 times (45 ml each) with 12 percent aqueous hydrochloric acid. The combined acid extracts were washed twice (15 ml each) with ether and the washings were combined with extracted (A), giving

(B). (B) was extracted 3 times (45 ml each) with 5 percent aqueous sodium hydroxide. The combined alkaline extracts were washed twice (15 ml each) with ether, and the washings were added to extracted (B), giving (C). (C) was washed with successive five ml portions of sodium-chloride saturated water until the lower layer was neutral to phenolphthalein. (C) was dried over anhydrous MgSO_4 and then reduced in volume to 30 ml on a Stedman column (80 cm) using a reflux ratio of 10:1. The 30 ml of ether was further concentrated to 2-3 ml on a micro spinning band column (25 cm) at a reflux ratio of 7:1. The 2-3 ml of ether solution was evaporated to 1.0 ml under a stream of nitrogen. Fifty μl of the 1.0 ml concentrate was removed and the weight of residue was obtained by evaporating under a gentle stream of nitrogen at room temperature for exactly 5 minutes². An aliquot of the 1.0 ml concentrate equivalent to 4.5-6.0 mg of residue was chromatographed using the Aerograph A-350 instrument³. The following conditions were employed: 10 ft Carbowax 20M column (0.25 in. dia., 20% W/W on acid-washed Chromosorb W); 75 ml helium per minute; injector and detector temperatures, 225° and 295°C, respectively; recorder speed, 0.75 in. per minute; and programming rate,

6° per minute for the range 70°-240°C followed by isothermal operation at 240°C for 30 minutes. Pertinent peak areas were measured with a planimeter using appropriate baseline corrections for background elution. For each peak, the "equivalent peak area" (EPA) for the entire 1.0 ml of concentrate was calculated by

$$\text{EPA} = \frac{10^\circ \text{A}}{\text{VW}}$$

in which A was the measured peak area (cm^2), V was the volume (μl) of the injected aliquot and W was the calculated weight (g) of moisture-free tobacco used in the steam distillation. Moisture was determined by heating 3 g of tobacco for 3 hours at 100°C in a forced draft oven.

In the identification work and in the sample shown in Figure 2 this method was modified to include steam distillation of larger weights of tobacco and injection of larger amounts of concentrate.

Variables.—The following variables were studied before adoption of the above method: weight of tobacco, volume of collected distillate, *in vacuo* vs. atmospheric distillation, method of ether removal and chromatographic conditions (stationary phase, carrier gas flow rate, programming rate, initial column temperature and weight of sample injected). Some of these variables were found to influence the observed quantitative but not the qualitative composition of a given tobacco. The selected conditions for the quantitative comparisons were chosen on the basis of anticipated optimal response of the various types and grades.

² The loss in weight during solvent removal decreases rapidly for 5 min. after which the rate of loss levels off markedly.

³ Use of a specific commercial product does not constitute endorsement by the United States Department of Agriculture over other products of a similar nature.

¹ Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

Results and Discussion

Differences between types and grades.—Preliminary study revealed that a large number of components was present in the steam-volatile substances. Figure 2 shows a chromatogram obtained from a 10 lb. sample of Turkish tobacco which was qualitatively similar to that obtained from a standard 100 g sample. Peak A was ether and peaks B to E originated from the solvent (ether) residue. The complexity of the mixture is well-illustrated by the number of minor peaks eluting at 100-190°C and the presence of discernible inflections on several peaks.

A comparison was made of the steam distillates of six representative commercial samples of various cigarette type tobaccos. Generally, the chromatograms of all types were qualitatively similar with respect to the numbered peaks in Figure 2; however, large quantitative differences were noted. Co-chromatography of the concentrates of all six samples in a single injection showed that these peaks chromatographed in an identical manner with the minor exceptions of peaks 9 and 12 which are discussed below.

Table 1 shows the ratios of the equivalent peak areas (EPA), total EPA and total weights of the steam-volatile neutral substances in the various tobacco types. Also included in this table are five samples of aged bright tobacco judged either "aromatic" or "aroma-deficient" by the commercial supplier. Peaks 13 and 14 were minor components which eluted slowly after peak 12 and are not shown in Figure 2. In most samples, peak 9 eluted as shown in Figure 2, but in four samples, a second peak eluted on the shoulder of peak 9. The main peak was designated 9A and the second peak, which eluted slower than 9A, was designat-

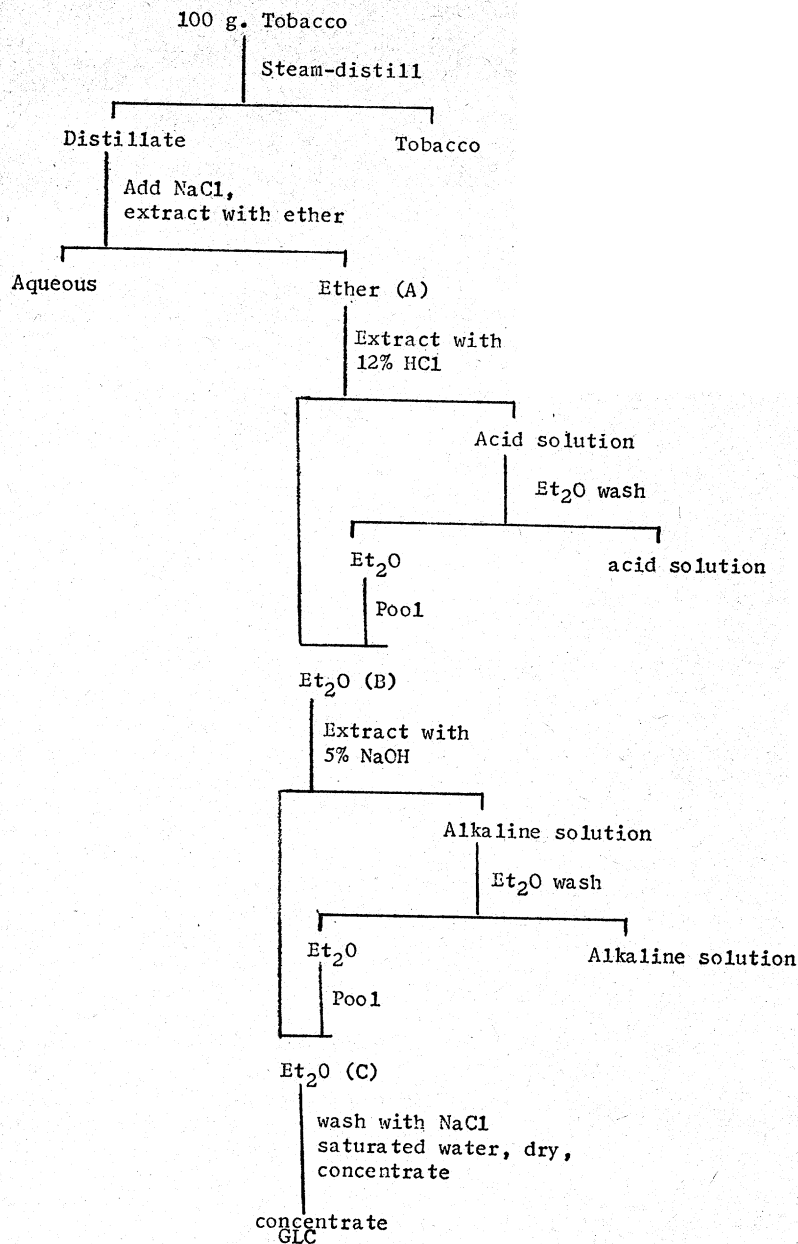


Figure 1. Separation of steam volatile neutral substances.

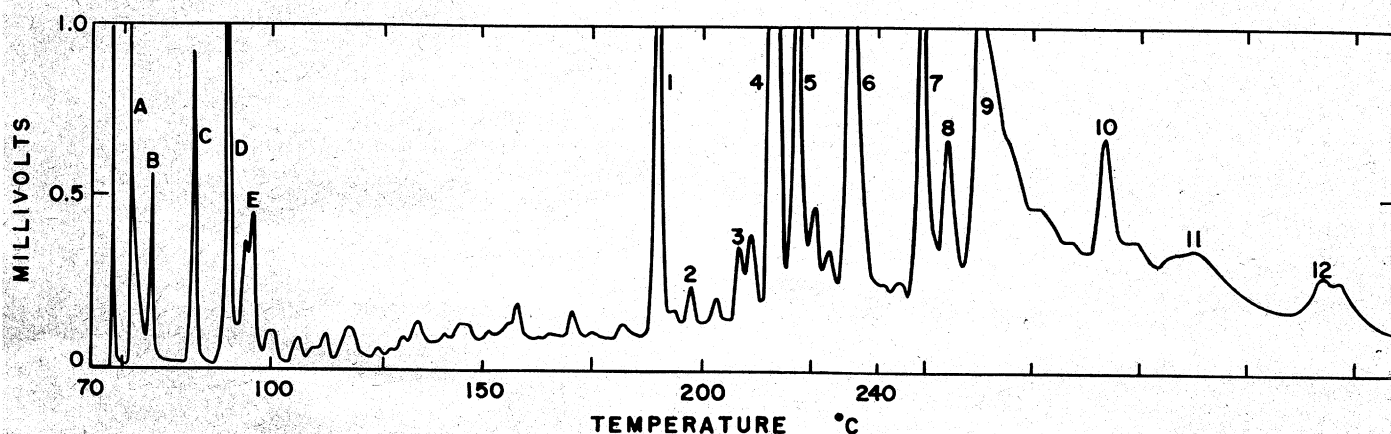


Table 1. Quantitative differences in certain steam-volatile neutral components of various tobacco types and grades.

	Total weight, neutrals (mg/100 g tob.*)	Ratios of equivalent peak areas (EPA) for indicated peak numbers**																Total EPA (cm ²)
		1	2	3	4	5	6	7	8	9A	9B	10	11	12A	12B	13	14	
Bright A	145	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	—	1.0	1.0	1.0	—	1.0	1.0	3387
Bright B	133	1.2	.65	.77	1.4	.98	.64	1.2	1.1	1.1	—	.73	2.3	1.7	—	1.7	1.7	3976
Burley	101	.04	.41	.23	.15	.72	2.1	1.4	7.7	1.7	—	2.7	2.3	1.6	.78	.96	.31	5205
Maryland	110	.18	.53	.29	.17	.86	5.6	2.9	3.2	1.6	—	8.2	1.5	3.3	1.4	.50	1.9	5560
Turkish (Smyrna)	176	.11	.53	.58	.62	1.4	5.8	2.0	5.5	1.5	—	3.1	2.0	.93	.26	.11	—	5128
Turkish (Samsun)	143	.19	1.2	.84	.87	2.4	7.7	3.6	11	2.0	—	7.7	3.7	3.0	.34	.72	1.8	7600
Aromatic A†	77	.67	.71	.71	1.1	.55	1.4	2.1	.22	1.5	.24	.27	.93	.46	—	.47	.32	4853
Aromatic B	56	.55	.59	.58	.84	.25	.68	1.5	.48	.88	.06	.50	.67	.23	—	1.7	.15	2765
Aromatic C	66	.56	.24	.71	.99	.26	1.1	2.2	.52	.90	.08	.36	.64	.40	—	.25	<.01	2986
Aroma- deficient A	59	.45	.53	.52	.73	.33	.78	1.4	.50	.88	.06	.50	.70	.23	—	.38	.25	2759
Aroma- deficient B	20	.83	.88	.65	.62	.31	.35	1.0	.07	.28	—	.32	.24	.21	—	.23	<.01	1156

* Moisture-free basis.

** EPA values (cm²) of the indicated peaks (in ascending order of peak number) of Bright A were as follows: 186, 17, 31, 201, 177, 119, 65, 46, 2248, 22, 87, 70, 53 and 65.

† The five aromatic or aroma-deficient samples were bright tobacco.

ed 9B. EPA ratios of both 9A and 9B were based on the EPA of 9A in the sample, Bright A. A comparable situation was observed with peak 12 and the evaluation was similarly handled using peak 12A of Bright A as the standard for ratio values.

The major component of the eluted substances was found in peak 9A. Among the six samples representing tobacco type, Turkish (Samsun) gave the highest total EPA; this tobacco contained more of the components in peak 5, 6, 7, 8, 9A and 11 than the other tobaccos. The bright samples yielded the lowest total EPA. Burley, Maryland and Turkish (Smyrna) gave intermediate total EPA. The four Turkish and bright samples were similar in that they contained more material in peaks 3, 4 and possibly 5 than burley or Maryland. There was a lack of correlation between the total weights of steam-volatile, neutral substances and total EPA in the six samples (see "Limitations" below).

Among the grades, the three aromatic samples gave higher total EPA values than the two aroma-deficient tobaccos, but the values for Aromatic B, Aromatic C and Aroma-deficient A were very similar. With the possible exception of peak 4, no components were present in larger amounts in all aromatic samples compared to all aroma-deficient tobaccos. Aroma-deficient B gave less material in peaks 6, 7, 8 and possibly 4 than the other four samples. Comparing Bright A and B with the five aromatic

or aroma-deficient grades, there were smaller amounts of peaks 1, 5, 8, 10, 12A and 14 in the grades. Four of the grades contained peak 9B.

Identification. Some effort was devoted to the identification of the above peaks. The available data are given in Table 2. All retention temperatures and co-chromatographic runs were obtained using programmed temperature gas chromatography on Carbowax 20M or silicone grease columns. Infrared spectra were determined on column effluents by examination of liquid films on micro sodium chloride plates. The qualitative functional group tests were performed using the method of Walsh and Merritt (1960) on column effluents. At least four criteria indicated that peaks 1, 4, 5, 6 and 9A were furfural, furfuryl alcohol, m-tolualdehyde, benzyl acetate, and neophytadiene, respectively; however, peak 9A probably contained other substances since small inflections were observed on the peak. Based on the infrared spectrum and chromatographic behavior, peak 9B may be isomerized neophytadiene (Stedman *et al.* 1960). Although very similar in chromatographic behavior, peaks 7 and 8 were not identical with β -phenethyl acetate and β -phenethyl alcohol, respectively. All of the above compounds have been previously reported in tobacco. Work is continuing on the identification of the unknown peaks.

Limitations. — The above steam

distillation method is an empirical procedure which does not yield comprehensive data on the volatile substances responsible for the odor of tobacco. Undoubtedly, steam distillation produces artifacts, such as furfural (Johnstone and Plimmer, 1959). Some higher boiling substances that may be steam-volatile to some extent, such as aliphatic paraffins (Onishi *et al.*, 1956), are not eluted in the chromatographic method which may account, in part, for the above lack of correlation between total weights of steam-volatile, neutral substances and total EPA. Lower boiling substances, such as acetaldehyde, and even some relatively high boiling compounds are lost in varying degrees during solvent removal (Stedman and Miller, 1962).

The above quantitative comparisons of unknown peaks on chromatograms are based on two assumptions: a linear relationship between peak area and concentration; and equivalent sensitivities of components in all peaks. A precise comparison would require that all peaks be identified and individual area-concentration relationships be established. The present survey was not intended to be a precise quantitative comparison and should be considered a preliminary examination of superficial qualitative and quantitative differences under limited conditions. Whether or not the chemical differences in Table 2 actually contribute in any way to the quality differences of type or grade is uncertain.

Summary

Using a standardized method for determining certain steam-volatile, neutral substances of tobacco by gas chromatography, a composition study was made of cigarette type tobaccos and of aromatic or aroma-deficient grades of bright tobacco. In general, the chromatograms of all tobaccos were qualitatively similar and quantitatively dissimilar. Bright tobacco contained less of the substances than burley, Maryland and Turkish. Turkish (Samsun) contained the most. No conclusive correlation was observed between the judged aromatic grades of the tobaccos and the quantities of certain components therein. Preliminary findings on the identities of some of the components are presented.

Acknowledgment

The authors wish to thank W. R. Bilinsky, S. F. Herb and H. C. Stetler of this Division for assistance and the following organizations for providing the tobacco samples: The American Tobacco Company, Brown and Williamson Tobacco Corporation and Philip Morris, Incorporated.

Literature Cited

Johnstone, R. A. W. and J. R. Plimmer, "The Chemical Constituents of Tobacco and Tobacco Smoke." *Chem. Revs.* 59: 885-936 (1959).
Onishi, I. et al., "Studies on the essential oils of Tobacco Leaves. I-XVI." *Bull. Agr. Chem. Soc. Japan* 19: 137-142 (1955), 19:

Table 2. Data on the identity of chromatographic peaks from the neutral, steam-volatile substances of tobacco.

Peak	Known compound	Retention temperature*		Co-chromatography*		Infrared spectrum*	Qualitative tests**
		C	SG	C	SG		
1	Furfural	+	+	+	+	+	+
2	Capric aldehyde	+	+				+
3	5-Methyl furfural	+		+			+
4	Furfuryl alcohol	+	+	+	+	+	
5	m-Tolualdehyde	+	+	+	+		+
6	Benzyl acetate	+	+	+	+		+
9A	Neophytadiene	+	+	+		+	

* C = Carbowax 20M column, SG = silicone grease column. + = responses of unknown peak and known compound were identical.

** Peaks 1, 2, 3 and 5 gave positive carbonyl (2,4-dinitrophenylhydrazine) tests and peak 6 gave a positive ester (hydroxamic acid-ferrous ion) test in the functional group reactions (see text).

143-147 (1955), 19: 149-152 (1955), 20: 61-67 (1956), 20: 68-69 (1956), 20: 70-73 (1956), 21: 38-42 (1957), 21: 43-46 (1957), 21: 82-85 (1957), 21: 86-89 (1957), 21: 90-94 (1957), 21: 95-98 (1957), 21: 177-180 (1957), 21: 181-184 (1957), 21: 239-242 (1957), 22: 17-20 (1958).
Onishi, I., H. Tomita and T. Fukuzumi, "Studies on the Essential Oils of Tobacco Leaves. IV. Neutral Fraction." *Bull. Agr. Chem. Soc. Japan* 20: 61-7 (1956).
Stedman, R. L. and R. L. Miller, "Some Pitfalls in Studies Related

to Gas Chromatography." *J. Chromatog. (in press)*.
Stedman, R. L., A. P. Swain and W. Rusaniwskyj, "Isomerization of Terpenoid Hydrocarbons by Acid-washed Alumina." *J. Chromatog.* 4: 252-3 (1960).
Walsh, J. T. and C. Merritt, Jr., "Qualitative Functional Group Analysis of Gas Chromatographic Effluents." *Anal. Chem.* 32: 1378-81 (1960).
Weybrew, J. A. and R. L. Stephens, "Survey of the Carbonyl Contents of Tobacco." *Tobacco Science* 6: 53-57 (1962).